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(54) Title: PROCESS FOR PRODUCING ALGINATES HAVING IMPROVED PHYSICAL PROPERTIES, AND THE USE OF SAID ALGINATES

(57) Abstract

Process for producing alginates having improved physical properties, by the inoculation of alginates derived from brown algae or bacteria, with an enzyme preparation such as a mannuronan-C-5-epimerase preparation from *Azotobacter vinelandii.* The modified alginates are used for immobilizing enzymes, cell organelles or cells as well as for the microencapsulation of biocatalysts.

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Process for producing alginates having improved physical properties, and the use of said alginates.

The present invention relates to the preparation of alginates having improved physical properties, especially with respect to the formation of gels having inorganic or polyvalent organic ions. Said modified alginates are intended to be used for immobilizing and encapsulating enzymes and/or cells for use in biotechnological processes.

Using alginate gels as an immobilizing material has several deficiencies, two of them being:

1) Calcium alginate gels are destabilized by compounds having affinity for calcium, for instance EDTA, citrate, lactate and phosphate, as well as high concentrations of cations such as Na^+, K^+ and Mg^{++};

2) Alginates currently used have a high degree of chemical heterogenity and provide gels having pores of such great size that proteins - enzymes and other macromolecules - can leach out, at the same time as the size distribution of the pores is difficult to control.

Alginate is the most important structural polysaccharide in marine brown algae and is used for several industrial purposes wherein the properties of the polymer are utilized as a polyelectrolyte - for instance for gel formation and thickening purposes - and also for its water and ion binding capacity.

The purpose of the present invention, thus, is to prepare alginates having physical properties satisfying the requirements for increased gel strength and stability and better controllable pore size.

Chemically seen, alginate is a polyuronide built up from two uronic acids, viz., D-mannuronic acid (M) and the C-5-epimer L-guluronic acid (G). They are arranged in such fashion that the polymer is further built up from three types of sequence: (G)-rich sequences called G-blocks, (M)-rich sequences called M-blocks and alternating structure symbolized by (MGMGMG).

The alginate's ability to form a gel by ionic binding, and the properties of said gel depends both on the relative content of the two uronic acids and on the distribution of the guluronic acid units along the chain. A high content of (G)-blocks yields, for instance, an alginate with great gel-forming capacity, which, technically seen, is a valuable property of the polymer.

The present invention is based on the following:

The alginate is synthesized in the alga as polymannuronic acid and is thereafter modified by an enzyme, mannuronan-C-5epimerase, which converts D-mannuronic acid residues into L-guluronic acid residues within the chain. When said enzyme affects the alginate, both the relative content and the uronic acid sequence will be changed and, consequently, its physical properties.

Thus, the invention relates to a process for producing alginates having improved physical properties such as increased gel strength, by using enzymatic modification on a polymeric level. The process is characterized in that alginates derived from brown algae or bacteria are inoculated with an enzyme preparation.

As such an enzyme preparation is preferably used a C-5-epimerase preparation, more preferably an alginate lyase-free mannuronan-C-5-epimerase produced from the earth bacterium Azotobacter vinelandii.

The present invention also comprises the use of the thus modified alginates for immobilizing enzymes, cell organelles and cells by entrapment in gels of alginate or alginate having suitable cations, as well as by immobilizing biocatalysts by encapsulation in alginate polycation microcapsules.

The mannuronan-C-5-epimerase may be isolated from cultures from the earth bacterium Azotobacter vinelandii, which produces both alginate and epimerase extracellularily. The fact that the enzyme is extracellular is a great advantage in the isolation process, and it also indicates that the enzyme may function freely in solution

independent on intracellular factors, which is favourable to a technical exploitation of the invention.

The use of immobilized enzymes as catalysts has obtained still greater importance in industry and will, in the years to come, become one of the most important expansion areas for biotechnology. Immobilized enzymes are often more stable, but first and foremost, they are easier to handle than free, soluble enzymes and may be used in continuous processes.

In addition to immobilizing simple enzymes there has also been developed techniques for immobilizing whole cells. The cells may serve as carriers for a single enzyme, such that isolation of the enzyme is unnecessary before immobilizing, or several enzymes may also be used in the cell in order to catalyse multistep processes (for instance synthesis of hormones, proteins, etc.).

We have tried out the epimerizing of a plurality of high polymer alga and bacterium alginates having varying block structures and formulation, and the conclusions are that all of the alginates can be epimerized to a substantial degree. The epimerization degree varies from 60 to 90 percent depending on the original block structure of the alginates and for some alginates this yields more than a doubling of the gel strength measured in 2 percent homogenous Ca-alginate gels.

Examples

Example 1

Sodium alginate derived from Laminaria digitata, in an amount of 0.07% by weight, was dissolved in cationic buffer, 0.05M collidine pH 7.0 and Ca^{2+} 6.8mM. This was incubated with a lyase-free C-5-epimerase preparation from A. vinelandii at 30°C for 8 hours. The epimerization degree, measured by means of high solution n.m.r.-spectroscopy, shows an increase of the guluronic acid content from 41% to 69% (see the Table).

Example 2

Sodium alginate from Macrocystis pyrifera, in an amount of 0.07% by weight, was dissolved in cationic buffer, 0.05M collidine pH 7.0 and Ca^{2+} 6.8mM. This was incubated with a lyase-free C-5-epimerase preparation from A. vinelandii at 30°C for 8 hours. The epimerization degree, measured by means of high solution n.m.r.-spectroscopy, shows an increase of the guluronic acid content from 37% to 62% (see the Table).

Example 3

Sodium alginate from Laminaria hyperborea, in an amount of 0.07% by weight was dissolved in a cationic buffer, 0.05M collidine pH 7.0 and Ca^{2+} 6.8mM. This was incubated with a lyase-free C-5-epimerase preparation from A. vinelandii at 30°C for 8 hours. The epimerization degree, measured by means of high solution n.m.r.-spectroscopy, shows an increase of the guluronic acid content from 68% to 79% (see the Table).

Example 4

Sodium alginate from Laminaria digitata containing 40% guluronic acid is treated with C-5-epimerase from A. vinelandii at pH 7.0 and Ca^{2+} 0.68mM for 6 hours at 30°C. The modified alginate contains 63% guluronic acid. Gel strength measurements on homogenous 2% calcium gels show a gel strength of 3.8 N/cm^2 and 9.6 N/cm^2 in native and enzyme modified alginate, respectively (see Figure 1).

TABLE

Composition and GG content of the polymer before and after enzymatic modification measured by high solution ^{1}H-n.m.r.-spectroscopy

Source	Before epimerization			After epimerization		
	F_G	F_M	F_{GG}	F_G	F_M	F_{GG}
Laminaria digitata	0.41	0.59	0.25	0.69	0.31	0.54
Laminaria hyperborea	0.68	0.32	0.57	0.79	0.21	0.67
Macrocystis pyrifera	0.37	0.63	0.14	0.62	0.38	0.32
Elachistae sp.	0.68	0.32	0.64	0.89	0.11	0.85
Dichtiosyphon foenicula.	0.67	0.33	0.61	0.81	0.19	0.75
Ascophyllum nodosum	0.36	0.64	0.16	0.63	0.37	0.39
Azotobacter vinelandii deacetylated	0.45	0.55	0.41	0.69	0.33	0.54

Claims

1. A process for producing alginates having improved physical properties, for instance increased gel strength, comprising inoculating alginates derived from brown algae or bacteria with an enzyme preparation.

2. The process of claim 1, wherein said enzyme preparation is a C-5-epimerase preparation.

3. The process of claim 1 or 2, wherein said mannuronan-C-5epimerase preparation is derived from Azotobacter vinelandii.

4. The use of alginates modified by the process according to any one of the preceding claims, for immobilizing enzymes, cell organelles and cells by gel entrapment in alginate gels or microcapsules of alginate and polycations.

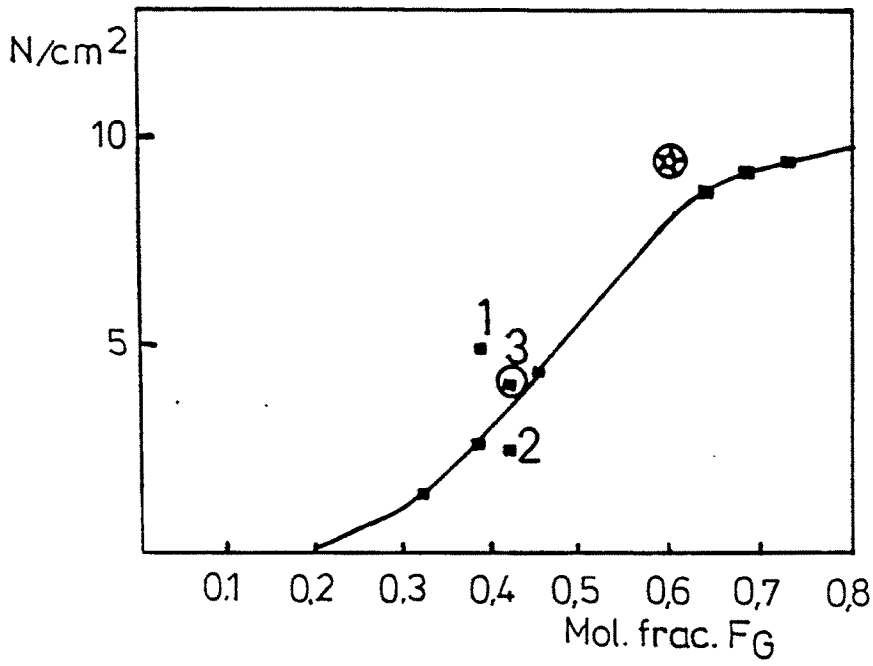

Fig.1

Gel strength measured on 2% alginate gels as a function of the guluronic acid content. 1. Macrocystis; 2. Acophyllum; 3. Laminaria digitata; ⊗ Enzyme treated with L. digitata.

INTERNATIONAL SEARCH REPORT

International Application No PCT/NO85/00080

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC 4

C 12 P 19/04, C 08 B 37/04, C 12 N 11/10 // (C 12 P 19/04, C 12 R 1:065)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	C 12 P 19/04; C 08 B 37/04; C 12 R 1:065; C 12 N 11/04; C 12 N 11/10
US Cl	536:3; 435:101; 195:31

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

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III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	Chemical abstracts, Vol 75, (1971) abstract No 564k, Carbohyd Res, 1971, 17(2), 297-308 (Eng.)	1-3, 4
Y	Derwents abstract No 13334 D/08, SU 742 434	4
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X,Y	Chemical abstracts, Vol 76, (1972), abstract No 31806r, Carbohyd Res 1971, 20(2), 225-32 (Eng).	1-3, 4
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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1986-04-04	1986 -04- 08
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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A	Chemical abstracts, Vol 95, 1981, abstract No 147178j, Nippon Suisan Gakkaishi 1981, 47(7), 889-93 (Eng).	1-4